Sub B1 cont.

d) amplifying the fragments that anneal to each other to generate at least one polynucleotide sequence encoding one or more protein motifs having altered characteristics as compared to the one or more protein motifs encoded by said parent polynucleotides.

Sub C1

2. A method as claimed in claim 1 wherein step (c) comprises adding primer sequences that anneal to the 3' and 5' ends of at least one of the parent polynucleotides under annealing conditions.

REMARKS

The June 29, 2001 Official Action and the references cited therein have been carefully reviewed. In view of the amendments presented herewith and the following remarks, favorable reconsideration and allowance of this application are respectfully requested.

The Examiner has rejected claim 1 under 35 U.S.C.§112, second paragraph as allegedly indefinite for the recitation of the term "optionally". Applicants respectfully submit that the term modifies the phrase "adding primer sequences that anneal to the 3' and 5' ends of at least one of the parent polynucleotides under annealing conditions" and not to remaining step d) of the claim. While not conceding the claim is in anyway indefinite, Applicants have amended the claim to delete the term "optionally" in order to advance prosecution of this application.

At page 2 of the Official Action, the Examiner has rejected claims 1-6 under the judicially created doctrine of obviousness double patenting for allegedly failing to be patentably distinct from claims 1-7 of US Patent No. 6,159,690.

Claims 1-6 stand rejected under 35 U.S.C. §102(e) as allegedly anticipated by US Patent 6,159,690 to Borrebaeck et al.

The Examiner has rejected claims 1-6 as allegedly unpatentable over US Patent 5,811,238 to Stemmer et al. and in view of Berger et al.

The rejections summarized above constitute the entirety of the rejections raised by the Examiner in the June 29, 2001 Official Action. No other issues are pending in the present application. Applicants respectfully submit that the claims as presently amended are in condition for allowance. Each of the above-noted rejections under 35 U.S.C. §112, second paragraph, the judicially created doctrine of double patenting, §102 and §103 is, therefore, respectfully traversed.

CLAIMS 1-6 ARE NOT PROPERLY REJECTED UNDER THE JUDICIALLY CREATED DOCTRINE OF OBVIOUSNESS DOUBLE PATENTING

The Examiner asserts that claims 1-6 of the present application are not patentably distinct from claims 1-7 of the '690 patent because both sets of claims are drawn to methods for generating a polynucleotide or population of polynucleotides from a parent polynucleotide sequences encoding one or more protein motifs. Applicants respectfully submit that the methods of the '690 patent for generating polynucleotide sequences are limited by the feature of step (b), namely "contacting the fragments with a <u>template</u> polynucleotide under annealing conditions". In contrast, in the methods of the present invention the single stranded fragments are annealed with each other, i.e., no template is added (See claim 1, step (c) of the present application. Thus the present claims are directed to a method wherein an essential element of the '690 method is omitted. Accordingly, Applicants submit that the methods presently claimed are not rendered obvious by the methods of the '690 patent and request that the rejection of claims 1-6 based on claims 1-7 of the '690 patent be withdrawn.

In further support of Applicants position, the results of certain experiments setting forth the unexpected advantage conferred by digesting single stranded DNA when

performing the method of claims 1-6 are provided below in connection with the arguments refuting the rejection under 35 U.S.C. § 103.

CLAIMS 1-6 ARE NOT ANTICIPATED BY US PATENT 6,159,690 TO BORREBAECK ET AL.

In order to constitute evidence of lack of novelty under 35 U.S.C. §102(b), a prior art reference must identically disclose each and every element of the rejected claim. In re Bond, 15 U.S.P.Q.2d 1566 (Fed. Cir. 1990). Applicants respectfully assert that the claims as presently amended are not anticipated by the disclosure in the '690 patent to Borrebaeck et al.

As stated above in connection with the rejection of the present claims based on the judicially created doctrine of obviousness type double patenting, the methods of the present invention involve annealing ssDNA fragments with each other. In contrast, the methods disclosed in the '690 patent require the presence of a template polynucleotide for annealing with the fragments prior to the amplifying step. Thus, as stated above, an essential component of the method recited in the '690 patent is omitted from the method of the present invention. In light of the foregoing, Applicants submit that claims 1-6 are novel over the disclosure of Borrebaeck et al. and request that the rejection under 35 U.S.C. §102(b) be withdrawn.

CLAIMS 1-6 AS AMENDED ARE NOT RENDERED OBVIOUS BY US PATENT 5,811,238 TO STEMMER ET AL. IN COMBINATION WITH BERGER

As a preliminary matter, Applicants confirm the Examiner's correct presumption that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made.

The criterion for determining obviousness under §103 is whether the prior art supplies some motivation or incentive to one of ordinary skill in the art to arrive at the invention as claimed. In re Dow Chemical Company, 5 U.S.P.Q. 2d 1929

(Fed. Cir. 1988). Obviousness cannot be established by combining teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. In re Fine, 5 U.S.P.Q.2d (Fed. Cir. 1988). Moreover, the teaching or suggestion supporting the desirability or the combination must be found in the prior art, not in applicant's disclosure. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992). Under these standards, neither of the cited references, considered singly or in combination, render obvious the invention as claimed in claims 1-6.

The '238 patent to Stemmer discloses several methods of generating variant polynucleotides in vitro, the methods comprising either:

1. fragmenting double-stranded parent polynucleotides and annealing the fragments formed thereby to each other (see column 5, line 51 over to column 6, line 36) or,

2. fragmenting double-stranded parent polynucleotides, denaturing the double-stranded fragments formed thereby and annealing the template polynucleotide (see column 6, lines 37-45).

Berger teaches hepaxoterministic restriction enzymes (which cleave double-stranded DNA at interupted palindromes) in site specific mutagenesis and the isolation of large intact DNA fragments.

In support of this obviousness rejection, the Examiner cites the paragraph at column 5, lines 51-67 of the '238 patent. However, in contrast to the methods of the present invention, which require digestion of single stranded DNA to produce single stranded fragments, it is explicitly stated in the cited paragraph that the method of Stemmer et al. comprises cleavage of double stranded polynucleotides to produce double stranded fragments. Likewise, the embodiments disclosed at column 6, lines 1-36 and all of the working examples also involve the cleavage of double stranded

polynucleotides. In light of the foregoing, Applicants respectfully submit that one of skill in the art would not be motivated upon the reading of the '238 patent to Stemmer to utilize single stranded DNA as the starting material in methods for generating polynucleotide variants as claimed in the instant application.

While the use of single stranded DNA is mentioned in passing at column 6, lines 37-45 of the '238 patent, it is clear from this passage of the description that the single stranded fragments are produced by cleavage of double-stranded polynucleotides, the resultant double-stranded fragments subsequently being denatured to produce the single-stranded fragments:

"…single-stranded fragments resulting from the cleavage and denaturation of the template polynucleotide…"

In addition, the described method requires annealing the single stranded fragments to a template polynucleotide. As discussed above, this is in direct contrast to the method of the present invention, wherein the single stranded fragments are annealed with one another. Inasmuch as the use of a template in the inadequately described single stranded method of the '238 Stemmer patent is clearly an integral feature of the method, Applicants submit that the skilled person would not be motivated to omit this template DNA from the method.

Berger describes methods for expanding the use of restriction endonucleases. Applicants submit that the Examiner has utilized hindsight reconstruction of Applicants invention in formulating the present obviousness rejection of claims 1-6 based on the combination of the '238 patent to Stemmer et al. and Berger. At the outset, the method of the present invention is directed to the use of exonucleases in methods to generate polynucleotide variants. Berger discloses the use of various endonucleases in site-directed mutagenesis methods. Indeed restriction endonucleases are enzymes which identify a defined palindrome-like sequence in double stranded

DNA and cleave both strands within the recognition site at precise locations. Berger is silent regarding the use of exonucleases in methods to generate polynucleotide variants. In summary, the skilled artisan would not be motivated to combine the teaching of Stemmer et al. and the teaching of Berger et al. to arrive at the method of the invention as presently claimed. Thus, the rejection of claims 1-6 as allegedly obvious over Stemmer and Berger is inappropriate and should be withdrawn.

The surprising results of Applicants experiments comparing the use of single stranded vs. double stranded DNA in the instant method supports Applicants position that the present claims are novel and non-obvious over the prior art cited by the Examiner. Applicants have compared the recombination frequencies between single stranded and double stranded DNA using the method presently claimed.

Three scFv genes were each amplified using the appropriate primers and standard PCR. The size of the bands was confirmed with agarose gel electrophoresis and the rest of the amplified PCR products were purified using Concert PCR purification kit (Gibco). The ds DNA from the three scFv were mixed in equimolar amounts and treated with BAL 31 according to the protocol (using 4, 20, or 100 U enzyme/ml) and samples were taken out at 0, 10, 30, and 50 minutes. The reactions were stopped with EDTA and heat treatment and purified using phenol/chloroform extraction and ethanol precipitation. Keeping each time point separate, the samples were subjected to reassembly PCR according to the protocol and cloned in pGEM. Eighteen clones from each time point were sequenced and the number and frequency of recombinations determined.

To repeat the above identified experiment using ssDNA (single stranded DNA), the three scFV genes were each amplified in two PCR reactions using the appropriate primer pairs and standard PCR procedure. The size of the bands was confirmed using agarose gel electrophoresis and the rest of the amplified PCR products purified using Concert PCR purification kit (Gibco). Single stranded DNA was obtained

using magnetic beads according to the protocol, achieving three sense strands and three antisense strands. The sens strands and the antisense strands, respectively from the three scFV were mixed in equimolar amounts and treated with BAL31 according to the protocol (using 1.25 or 11 U enzyme/ml and samples were taken out at 0, 10, 30, and 50 minutes. The reactions were stopped with EDTA and heat treatment and purified using phenol chloroform extraction and ethanol precipitation. Keeping each time point separate, but mixing sense and antisense strands, the samples were subjected to reassembly PCR (for this reassembly 60 ng DNA is used) and amplification PCR according to the protocol provided by the manufacturer. The resulting product was then cloned into pGEM. Eighteen clones from each time point were sequenced and the number and frequency of recombinations were determined.

The results revealed that the highest frequency of recombination using ds DNA was achieved using 20 U enzyme/ ml and treating for 10 minutes. This gave 39% of the clones with one crossover and 17% of the clones with 2 crossovers. In contrast, the highest frequency of recombination giving one crossover using ssDNA was achieved using 11 U enzyme/ml and treatment for 10 minutes. 59% of the resulting clones had one cross over. The highest frequency of recombination giving two crossovers using ssDNA was achieved using 1.25 U enzyme/ml and treatment for 30 minutes. 20% of the resulting clones had two crossovers.

Further supporting the superiority of the presently claimed method are experiments showing the improved control of fragment size achieved which utilizing exonucleases such as BAL 31 and exonuclease VII as claimed herein. Both enzymes cleave off one nucleotide at a time, either from the 5′ end or from the 5′ and 3′ ends. The reaction can be stopped using EDTA or heat inactivation. This means that fragments of all possible sizes, differing by only one nucleotide, can be obtained. In a typical experiment, about 300 ng DNA is isolated at several consecutive time points of BAL31 treatment for example at 0, 10 and 30 minutes of incubation, the

reaction being stopped at the indicated time points. Following treatment the fragments are resolved by agarose gel electrophoresis. With increasing amounts of time, increasing amounts of DNA are digested. Corresponding gel histograms show multiple peaks which indicate different conformations of ssDNA. Peaks of distinct sizes were obtained. The data reveal that as the peak corresponding to larger fragments is decreasing, peaks corresponding to fragments of a smaller size are concomitantly increasing. An HPLC system is also being employed to resolve the fragments with greater accuracy.

The foregoing data clearly show the superior and unexpected results achieved using ssDNA as the starting material and exonuclease digestion in methods for creating polynucleotide variants.

Applicants are also submitting, in a separate paper, an Information Disclosure Statement and PTO form 1449. Also being submitted is the appropriate fee for filing the statement after a first Official Action on the merits but prior to the indication of allowable subject matter.

The present communication is completely responsive to the issues raised in the Official Action of June 29, 2001. Applicants believe that the claims as they stand are in condition for ready allowance. In the event the Examiner is not persuaded as to the allowability of any claim, and it appears that any outstanding issues may be resolved through a telephone interview, the Examiner is requested to telephone the undersigned attorney at the phone number give below.

Respectfully submitted,
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Enclosures: Marked up copy of amended claims

Marked up draft of claims

1. A method for generating a polynucleotide sequence or population of sequences from parent single stranded polynucleotide sequences encoding one or more protein motifs, comprising the steps of:

a) providing single stranded DNA constituting plus and minus strands of parent polynucleotide sequences;

b) digesting the single-stranded polynucleotide sequences with a<u>n</u> [nuclease other than DNAase I] <u>exonuclease</u> to generate populations of single stranded fragments;

c) contacting said fragments generated from the plus strands with fragments generated from the minus strands [and optionally, adding primer sequences that anneal to the 3' and 5' ends of at least one of the parent polynucleotides under annealing conditions];

d) amplifying the fragments that anneal to each other to generate at least one polynucleotide sequence encoding one or more protein motifs having altered characteristics as compared to the one or more protein motifs encoded by said parent polynucleotides.

2. A method as claimed in claim 1 wherein [the nuclease other than DNAase I is an exonuclease] <u>step (c) comprises adding primer sequences that anneal to the 3' and 5' ends of at least one of the parent polynucleotides under annealing conditions.</u>